DATA EVALUATION RECORD

1. **CHEMICAL**: NTN 33893 129099
   Shaughnessy No. ~~129059~~

2. **TEST MATERIAL**: Technical NTN 33893, 97.4%.

3. **STUDY TYPE**: Acute Toxicity Test for Freshwater Fish, Bluegill Sunfish (Lepomis macrochirus).

4. **CITATION**: Bowman, J. and J. Bucksath. 1990. "Acute Toxicity of NTN 33893 to Bluegill (Lepomis macrochirus)". Analytical Bio-Chemistry Laboratories, Inc. Aquatic Toxicology Division, 7200 East ABC Lane, P.O. Box 1097, Columbia, Missouri 65205. Laboratory Report No. 37860. Submitted by Mobay Corporation, Research and Development Department, P.O. Box 4913, Kansas City Missouri 64120. US EPA MRID No. 420553-14.

5. **REVIEWED BY**:

   Dana Lateulere, Biologist
   Ecological Effects Branch
   Environmental Fate and
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   Signature: Dana Lateulere
   Date: 12/2/91

6. **APPROVED BY**:

   Ann Stavola, Section Head, 5
   Ecological Effects Branch
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   Effects Division

   Signature: Ann Stavola
   Date: 1/29/92

7. **CONCLUSIONS**: This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test to freshwater fish, bluegill sunfish. An LC50 was not determined due to low mortality in the highest concentration tested and no mortality in any of the lower concentrations. Therefore the LC50 is >105 mg/L which classifies NTN 33893 as practically non-toxic to bluegill sunfish. The LOEC is 42 mg/L based on abnormal behavior at this level. The NOEC is 25 mg/L.

8. **RECOMMENDATIONS**:

9. **BACKGROUND**: This study was submitted as part of registration and EUP requirements.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Bluegill (ABC Lot# 6489) used in the test were obtained from Osage Catfisheries in Osage Beach, Missouri. All fish were from the same source and year. All test fish were held in well water on a 16 hour daylight photoperiod and observed for approximately 22 weeks prior to testing. The bluegill used for this experiment had a mean weight of .46 g and a mean standard length of 27 (+/-2) mm. This gave a test chamber loading biomass of .31 g/L for the definitive study.

B. Test System: Fish were acclimated to the test temperature and to the soft blended dilution water for 48 hours prior to testing. Fish were not fed during the acclimation and test periods.

The static fish bioassay was conducted in five gallon glass vessels containing 15 liters of soft blended water which was equivalent to a depth of approximately 29.9 cm. The test vessels were placed in a waterbath and temperature was maintained at approximately 22°C.

C. Dosage: A range-finding test was conducted to determine the concentrations for the definitive study. Nominal concentrations were: 0, 0 (DMF), 16, 27, 45, 75, and 125 mg/L. The measured concentrations were: 0, 0, 14, 25, 42, 68, and 105 mg/L.

D. Design: Ten fish per concentration were selected for the definitive bioassay; also included was a dilution water control and solvent control chamber. The fish were added to the test chambers in an impartial manner within 30 minutes after addition of test material. All test organisms were observed once every 24 hours for mortality and abnormal effects during the 96-hour exposure duration. Any dead fish were removed from the test chambers after each 24-hour observation.

The soft blended water used as dilution water in this study was prepared by blending naturally hard well water with well water that had been demineralized by reverse osmosis. This soft water was prepared to yield a total hardness of 40-48 mg/L as $CaCO_3$.

Temperature, dissolved oxygen and pH were measured in the control, solvent control, low, middle and high test concentrations with live fish at 0, 48, and 96 hours. No aeration was necessary during the study. The lighting was maintained on a 16 hour daylight photoperiod. The light intensity in the test area was an average of 29 footcandles. The water bath temperature was monitored continuously during the study.

E. **Statistics:** A computerized program calculated the LC50 and its 95% confidence limits using the binomial, moving average and probit methods. Three different methods of analyzing the data were used since no one method of analysis is appropriate for all possible sets of data. The method of calculation selected in this report was that which gave the narrowest confidence limits for the LC50.

12. **REPORTED RESULTS:** The 24, 48, 72 and 96 hour LC50 values for bluegill exposed to NTN 33893 were all >105 mg/L. The LC50 was shown to be greater than the water solubility limits of the test material. The 96-hour no-observed-effect concentration was estimated to be 25 mg/L, based on the lack of morality or observed abnormal effects at this concentration. The abnormal effects of dark discoloration, fish on the bottom of test chamber, erratic swimming, surfacing, quiescence, rapid fin movement and/or labored respiration were observed in the 42, 68, and 105 mg/L test concentrations. Thirty percent mortality was observed in the 105 mg/L test concentration at 96 hours.

The dissolved oxygen concentrations ranged from 4.1 to 8.3 mg/L during the test; this was considered adequate for testing. The pH values ranged from 7.3 to 7.8.

Surface film and precipitate on the bottom were noted in the 42, 68, and 105 mg/L test chambers at 0-hour. The surface film was no longer visible in the 42 mg/L chamber after 48 hours. The precipitate in the 68 and 105 mg/L chambers decreased with time but remained visible throughout the 96 hour test period.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 96-hour LC50 for bluegill (Lepomis macrochirus) exposed to NTN 33893 was >105 mg/L. The LC50 was shown to be greater than the water solubility limits of the test material. The NOEC in this study was 25 mg/L.

Quality Assurance Inspection was conducted for compliance verification by the Quality Assurance Unit. It was also stated that this study was conducted in compliance with the Good Laboratory Practice Standards, 40 CFR Part 160.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were in accordance with Subdivision E, and SEP guidelines except for the following deviations:

- Temperature is to be monitored and recorded every six hours when controlled by a water bath; temperature was recorded every 48 hours.

B. **Statistical Analysis:** The reviewer used Toxanol to determine the 96 hour LC50 value. The NOEC was estimated based on reported results of mortalities and abnormal behavior.

C. **Discussion/Results:** An LC50 was not determined due to low mortality in the highest concentration tested and no mortality in any of the lower concentrations. Therefore the LC50 is >105 mg/L which classifies NTN 33893 as practically non-toxic to bluegill sunfish. The LOEC is 42 mg/L based on abnormal behavior at this level. The NOEC is 25 mg/L.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale:

(3) Repairability:

TABLE 5

Mortality and Behavioral Observations During the Acute Toxicity Test of NTN 33893 with Bluegill (Lepomis macrochirus)

Mean Measured Conc. mg/L (ppm)	Number Placed in Test	24-Hour		48-Hour		72-Hour		96-Hour	
		Mort.	Observations	Cum. Mort.	Observations	Cum. Mort.	Observations	Cum. Mort.	Observations
Control	10	0	10 N	0	10 N	0	10 N	0	10 N
Solvent Control	10	0	10 N	0	10 N	0	10 N	0	10 N
14	10	0	10 N	0	10 N	0	10 N	0	10 N
25	10	0	10 N	0	10 N	0	10 N	0	10 N
42	10	0	9 N; 1 SUR/Q	0	1 SUR/RF; 3 RF; 6 N	0	1 SUR/RF; 7 RF; 2 N	0	1 RF/DK; 1 SUR/RF; 8 N
68	10	0	4 DK/RF; 3 DK/OB/Q/RF; 3 DK/SUR/Q/RF	0	2 DK/RF; 1 OB/Q/DK; 6 DK/SUR/RF; 1 OB/Q/RF/DK	0	3 RF; 2 OB/Q/RF; 4 SUR/DK/RF; 1 OB/Q/RF/DK	0	3 DK/RF; 2 OB/DK/RF; 5 SUR/DK/RF
105	10	0	1 DK/Q/RF; 7 DK/OB/Q/RF; 2 DK/SUR/RF/ES	0	1 DK/RF; 7 DK/OB/Q; 2 DK/SUR/ES/RF	0	1 DK/RF; 7 DK/OB/Q/RF; 2 SUR/DK/RF/LR	3	3 OB/DK/RF/Q; 4 SUR/RF/DK/ES

Key to Observations: N = Normal; Q = Quiescent; SUR = Surfacing; OB = On Bottom of Test Vessel; DK = Dark Discoloration; LR = Labored Respiration; RF = Rapid Fin Movement; ES = Erratic Swimming.

TABLE 6

Water Quality Measurements During the Acute Toxicity Test of NTN 33893 with Bluegill (Lepomis macrochirus)

Mean Measured Concentration (mg/L)	Water Quality								
	0-Hour			48-Hour			96-Hour		
	Temp.[a] °C	D.O.[b] mg/L	pH[c]	Temp. °C	D.O. mg/L	pH	Temp. °C	D.O. mg/L	pH
Control	22	8.3	7.7	22	5.9	7.4	22	4.1	7.4
Solvent Control	22	8.3	7.8	22	6.5	7.4	22	5.1	7.4
14	22	8.3	7.8	22	6.4	7.5	22	5.4	7.4
25									
42	22	8.3	7.8	22	6.1	7.4	22	4.6	7.3
68									
105	22	8.1	7.8	22	5.9	7.5	22	5.2	7.4

[a] Temperature measured using a mercury thermometer.

[b] Dissolved oxygen concentrations - Dissolved Oxygen Probe (YSI Model 54).

[c] pH - pH Probe (Corning Model 476182) used with a Corning Model 125 pH and mV meter.

Note: Dissolved oxygen saturation corrected for altitude at the test temperature of 22°C is 8.4 mg/L.